Immune response during lactation after anti-SARS-CoV2 mRNA vaccine

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Key Points:

Question: What is the immunologic response to anti-COVID-19 mRNA-based vaccines during lactation and does vaccination induce secretion of IgA antibodies into human milk?

Findings: In a cohort of 23 lactating individuals who were vaccinated against SARS-CoV-2, we found significantly increased levels of anti-SARS-CoV2 IgG and IgM antibodies in plasma, as well as anti-SARS-CoV2 IgA in human milk.

Meaning: Lactating individuals receiving anti-COVID-19 vaccines transfer antibodies to their infants and given the long-term health benefits of breastfeeding for the maternal-infant dyad, lactating individuals should be encouraged to continue to breastfeed after vaccination.

Abstract:

Importance: Data regarding efficacy and safety of anti-COVID-19 mRNA vaccines during lactation is needed to address vaccination guidelines, ease vaccine hesitancy concerns, and inform public health strategies for this population.

Objective: To determine whether anti-COVID-19 mRNA-based vaccines administered during lactation ellicit an immune response or the transfer of anti-SARS-CoV2 antibodies into human milk.

Design: Plasma and milk samples were collected from a prospective cohort of lactating individuals who received the mRNA-based vaccines for COVID-19 and from individuals who recovered from COVID-19 infection.

Setting: Ambulatory or during postpartum hospitalization.

Participants: We report results from lactating participants who received the mRNA-1273 (Moderna, n=9) or the BNT162b2 (Pfizer, n=14) vaccine or recovered from natural SARS-CoV-2 infection (n=3).

Interventions and Exposures: Anti-COVID-19 mRNA vaccination (BNT-162b2 and mRNA-1273) or natural SARS-CoV-2 infection.

Main Outcome(s) and Measure(s): Plasma and milk samples were collected from lactating individuals before first vaccine dose, on the day of the second dose, and 4 weeks after the second dose. Maternal plasma was evaluated for vaccine-derived IgM and IgG antibodies. Human milk was evaluated by ELISA for vaccine-induced IgA antibodies specific for SARS-CoV-2.

Results: Twenty-three lactating individuals were recruited for this study. Levels of IgG and IgM were significantly increased in plasma samples on the day of the second vaccine dose (post vaccine 1), when compared to pre-vaccine samples. In addition, plasma IgG levels 4 weeks after second vaccine dose were significantly higher than plasma IgG levels pre-vaccine or on the day of the second dose. In addition, our results show transfer of anti-SARS-CoV2-Receptor Binding Domain (RBD) IgA antibodies to human milk, 3-4 weeks after each dose of the COVID-19 mRNA vaccines (BNT-162b2 and mRNA-1273). The levels of anti-SARS-CoV2-RBD IgA antibody in milk of vaccinated individuals were not significantly different from levels among participants who experienced SARS-CoV-2 infection.

Conclusions and Relevance: Administration of anti-COVID-19 mRNA vaccines during lactation leads to increased anti-SARS-CoV2 IgM and IgG levels in the plasma of lactating mothers and increased anti-SARS-CoV2-RBD IgA levels in human milk. Lactating women who receive the vaccine should consider continuing to breastfeed their infant human milk to allow transfer of anti-SARS-CoV-2 IgA antibodies to the neonate. Additional studies are needed to evaluate the effect of these vaccines on lactation outcomes and infant health.

Introduction:

Several countries have approved the use of anti-COVID-19 mRNA based vaccines under an emergency use authorization[1]. However, the Phase III clinical trials[2,3] of these vaccines excluded lactating individuals and therefore there is no available data on the efficacy and safety of these vaccines on lactating individuals and their infants. Currently, the World Health Organization (WHO) recommends lactating individuals to obtain the vaccine when eligible and does not advise cessation of breastfeeding following receipt of the vaccine[4]. The Academy of Breastfeeding Medicine (ABM) states that there is little plausible risk that vaccine lipid particles would enter the blood stream and be present in breast tissue, and that nanoparticles or mRNA would be transferred to milk [5]. We recently showed that mRNA from either the Pfizer- BioNTech (BNT-162b2) or Moderna (mRNA-1273) vaccines were not detected in human milk samples collected from vaccinated individuals 4-48 hours after vaccines[6]. However, despite these recommendations and evidence, some mothers have declined vaccination, chosen or been advised to discard their milk for up to 72 hours post vaccine, or decided to stop breastfeeding earlier than planned due to the lack of solid evidence about the effect of the mRNA vaccine on human milk and infant health. An important benefit of human milk is the presence of IgA antibodies that provide passive immunity to the infant[7,8]. In addition, anti-SARS-CoV2 antibodies were recently shown to be present in milk from lactating women that were infected with SARS-CoV2[9,10]. However, there are no data on whether the COVID-19 mRNA vaccines induce the secretion of anti-SARS-CoV-2 antibodies into human milk. Here, we show that milk samples collected 3-8 weeks post BNT-162b2 and mRNA-1273 vaccination contain significantly higher levels of anti-SARS-CoV2-RBD IgA antibodies when compered to pre-vaccine samples. Importantly, we show significant increases in anti-SARS-CoV2 IgG and IgM levels post vaccine in plasma of lactating individuals.

Methods:

The University of California San Francisco (UCSF) institutional review board approved the study (20-32077, 20-30410). Informed consent was obtained from all participants. Detailed methods are described in the Supplemental Materials. Briefly, maternal plasma was analysed for anti-SARS-CoV-2 IgM and IgG antibodies, which were quantified through Pylon 3D automated immunoassay (considered positive if ≥ 50 relative fluorescence units (RFU)). Blood samples were collected up to 24 hours before first vaccine dose (n=7), up to 24 hours before second dose (n=12), and 4 weeks after second vaccine dose (n=14). At similar time points, human milk samples were collected fresh or frozen immediately after expressing. For some participants, additional milk samples were collected weekly post vaccine. Human milk samples were assayed by ELISA for IgA antibodies specific to SARS-CoV-2 RBD protein.

Results:

We analysed 7 pre-vaccine, 12 post first vaccine administration, and 14 post second vaccine administration plasma samples from vaccinated lactating individuals. We found a significant increase in anti-SARS-CoV-2 IgM and IgG antibodies post first vaccine administration and post second vaccine administration, when compared to pre-vaccine samples (**Figure 1A and 1B**). Furthermore, anti-SARS-CoV-2 IgG levels at 4 weeks after the second dose were significantly higher (P value <0.0001) compared to samples collected at 3-4 weeks after first dose (collected on the day of second dose) (**Figure 1A)**. In contrast, anti-SARS-CoV-2 IgM levels were not significantly higher 4 weeks after the second dose compared to samples collected after the first dose (**Figure 1B).**

In addition, we found significantly higher levels of IgA antibodies specific to SARS-CoV-2 RBD protein in human milk samples collected after the first dose of both vaccines (**Figure 2A and 2B**). Seventeen out of 19 milk samples analysed at the day of the second vaccine dose were

positive for anti- SARS-CoV-2 IgA antibodies. Thirteen out of the 15 milk samples that were analysed 4 weeks after the second dose were positive for anti-SARS-CoV-2 RBD IgA (**Figure 2A and 2B)**. We also analysed 3 milk samples collect from lactating individuals who were previously infected by SARS-CoV-2 virus. Anti-SARS-CoV-2 RBD IgA antibodies levels in milk samples from vaccinated individuals were not significantly different from samples collected after natural SARS-CoV-2 infection (**Figure 2A and 2B,** right columns**)**

When analyzing serial samples collected from 0 to 64 days after first vaccine administration from 10 patients, we found variation in anti-SARS-CoV-2 RBD IgA antibodies levels in milk between individuals (**Figure 2C and 2D**). In contrast to the significant increase in plasma IgG after the second dose (**Figure 1A**), milk IgA levels largely remained stable 4 weeks after the second dose (**Figure 2C**).

Conclusion:

We show here that administration of anti-COVID-19 mRNA vaccines during lactation leads to a significant increase in anti-SARS-CoV2 IgM and IgG levels in the plasma. Consistent with results from previous studies that showed reduced IgM levels 28 days after COVID-19 infection,[11] our results show that IgM levels 4 weeks post second dose administration were not significantly higher compared to their levels after the first dose. Furthermore, our data clearly demonstrate increased anti-SARS-CoV2-RBD IgA levels in human milk consistent with levels found after SARS-CoV-2 infection. Taken together, our findings suggest that anti-COVID-19 vaccination is beneficial for lactating individuals and may also protect their infants.

Our results provide information needed to inform the care of lactating individuals and their infants[12]. Although there is expert consensus of minimal or no potential risk for the infant from maternal anti-COVID-19 vaccination[13,14], no previous studies have demonstrated specific benefits during lactation. Our results now provide such evidence. Future studies are needed to

152 determine the degree of protection conferred by these IgA anti-SARS-CoV-2 antibodies. At the
153 present time, our findings suggest that lactating individuals who receive vaccination should
154 continue to breastfeed and chestfeed their infants in order to ensure transfer of anti-SARS-CoV-
155 2 IgA antibodies to their baby that may potentially protect against COVID-19, important even
156 during infancy[15]. Health care providers should continue to encourage lactating individuals to
157 breastfeed and chestfeed their infant during and following COVID-19 vaccination.

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Conflict of interest:

The authors declare no conflicts of interest.

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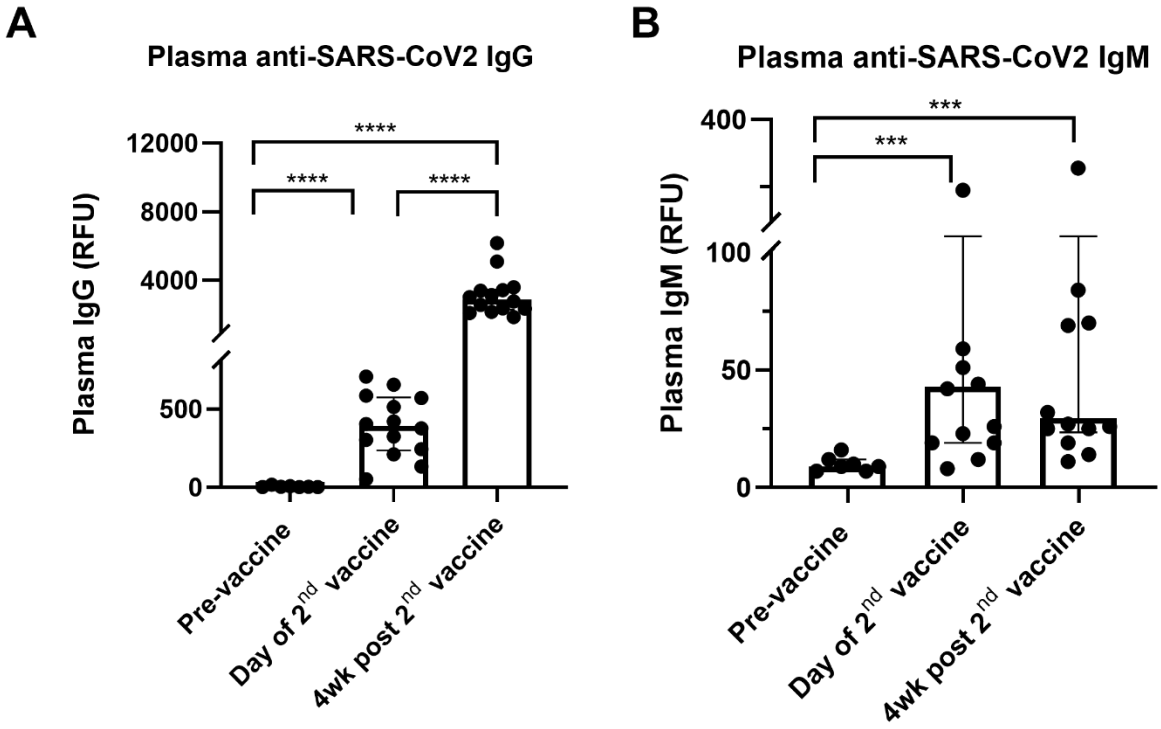
212 **Figure and legend:**



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215 **Figure 1: Elevated levels of plasma anti-SARS-CoV2 antibodies in COVID-19 mRNA**
216 **vaccinated lactating individuals.** Anti-SARS-CoV2 IgG (A) and IgM (B) levels in plasma of
217 lactating individuals post COVID-19 mRNA vaccine (n=18, RFU- relative fluorescent units,
218 positive cut-off >50 RFU-relative fluorescent units). Asterisks represents p-values: *= p-value
219 <0.05, **= p-value <0.01, ***= <0.001, ****= <0.0001 as determined by unpaired Mann-Whitney
220 test.

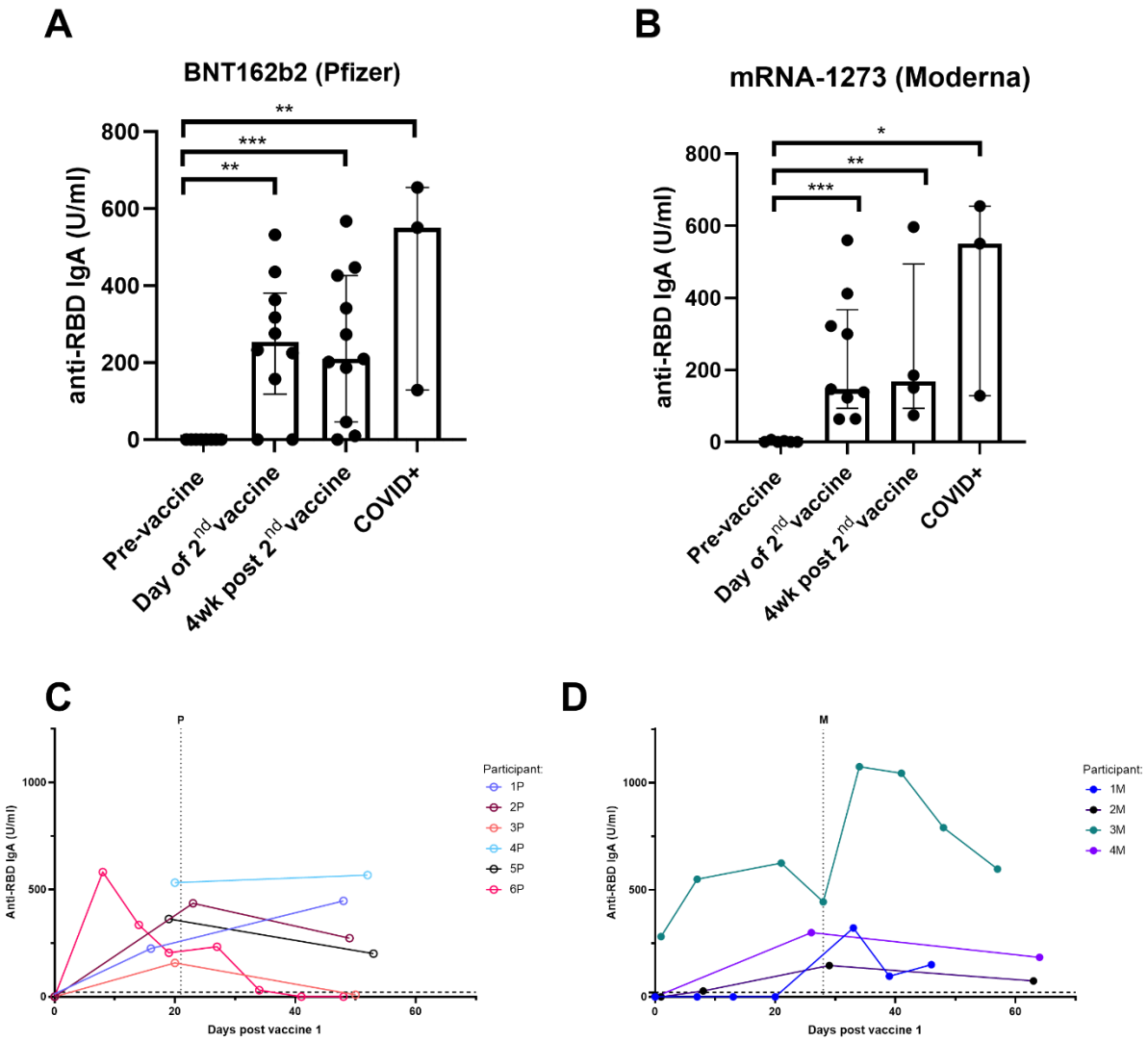

Figure 2: Elevated levels of milk anti-SARS-CoV2 IgA antibodies in COVID-19 mRNA vaccinated lactating individuals. Milk samples from individuals receiving BNT-162b2 (n=14) (A) and mRNA-1273 (n=9) (B) anti-COVID-19 vaccines were analysed for anti-SARS-CoV2 IgA antibodies using ELISA. Asterisks represents p-values: *= p-value <0.05, **= p-value <0.01, ***= <0.001 as determined by unpaired Mann-Whitney test. Anti-SARS-CoV2 IgA antibodies levels in milk at serial time points post first BNT-162b2 (C) and mRNA-1273 (D) vaccine dose administration. Dashed vertical lines represent

the timing of the second dose administration (P- BNT-162b2, M- mRNA-1273). Dashed horizontal lines represent 21.4 U/mL, values above this line were considered positive. Sample identification by participant number and type of vaccine observed (P- BNT-162b2, M- mRNA-1273). Note: first sample from M3 obtained 24h post first vaccine.